Environmental Health Perspectives
Vol. 29, pp. 71–79, 1979

N-Acetyltransferase Phenotype and Risk in Urinary Bladder Cancer: Approaches in Molecular Epidemiology. Preliminary Results in Sweden and Denmark

by G. M. Lower, Jr.,* T. Nilsson,† C. E. Nelson,† H. Wolf,‡ T. E. Gamsky,* and G. T. Bryan*

A variable but often significant proportion of urinary bladder cancer in urban areas can be attributed to occupational and cultural (cigarette smoking) situations associated with exposures to various arylamines. The variable *N*-acetylation of carcinogenic arylamines by human hepatic enzyme systems, the known genetic regulation and polymorphic distribution of this enzyme activity in humans, and the known enhanced susceptibility of individuals with the genetically-distinct "slow acetylator" phenotype to various arylamine toxicities, has prompted examination of possible correlations between *N*-acetyltransferase phenotype and urinary bladder cancer risk in rural and urban populations. In this context, *N*-acetylation is viewed as a component of detoxication pathways with respect to arylamine bladder carcinogenesis.

In preliminary utilizations of this approach, a population of urban urinary bladder cancer patients from Copenhagen, Denmark displayed a 13% excess ($p = 0.065$) of individuals with the slow acetylator phenotype (46/71 = 64.8%) when compared to a Danish control population (38/74 = 51.4%). These data are consistent with the possibility that arylamines may play an etiological role in bladder cancer in this locale and that slow acetylator individuals may be at higher relative risk (1.74) than rapid acetylator individuals. As 95% of patients reported histories of smoking, it was not possible to isolate and examine smoking factors.

In contrast, a population of rural urinary bladder cancer patients from Lund, Sweden, where bladder cancer incidence (20/100,000) (1971) is lower than in Copenhagen (43.8/100,000) (1968-72), no difference in slow acetylator distribution was observed between bladder cancer (80/115 = 69.6%) and Swedish control (79/118 = 66.9%) populations, indicating a relative lack of involvement of arylamines in the etiology of rural bladder cancer.

Populations of "spontaneous" bladder cancer patients would be expected to contain variable portions of disease related to arylamine exposure and would be less likely to display a detectable correlation than would an industrial population with documentable arylamine exposure. Consequently, confirmation of this hypothesis is being pursued by examination of industrial populations in an effort to obtain an empirical estimate of relative risk for slow and rapid acetylator phenotypes. These studies involve exposure-matched workmen both with and without bladder cancer.

Introduction

Since the initial clinical observations of bladder cancer victims in the German chemical dye industry by Rehn in 1895 (*1*), the induction of bladder cancer in dogs following administration of 2-aminonaphthalene by Hueper in 1938 (*2*), and the analytical epidemiological investigations of bladder cancer risk within the British dye industries by Case in 1953 (*3*), considerable direct evidence has been accumulated to implicate arylamines and metabolically related aryl nitrogen compounds in the genesis of human urinary bladder cancer. Thus, urinary bladder cancer is recognized as the first human

* Department of Human Oncology, University of Wisconsin Center for Health Sciences, Madison, Wisconsin 53706.
† Department of Urology, University Hospital, Lund, Sweden.
‡ Department of Urology, Hvidovre University Hospital, Copenhagen, Denmark.

neoplasm for which precisely-defined chemicals were suggested as causal agents and for which chronic exposure to a variety of occupational and environmental chemicals was suggested as a determining factor in a high percentage of disease incidence (*4*, *5*).

Those arylamines posing established carcinogenic hazard to humans include 2-aminonaphthalene, 4-aminobiphenyl, 4,4′-diaminobiphenyl, and 4-nitrobiphenyl utilized in various industrial processes (*4*, *5*) (Fig. 1). Such arylamines have found use as reagents in the preparation of various textile and hair dyes and plant pigments, as antioxidants in the preparation of rubber for the manufacture of tires and cables, and as curing agents in the preparation of various plastics. Similarly, a number of arylamines, such as 3,3′-dichlorobenzidine and 4,4′-methylene bis(2-chloroaniline) utilized as curing agents in the preparation of polyurethane elastomers, are potent bladder carcinogens in rodent or dog model systems and it is probable that these agents have human carcinogenic potential (*6*, *7*). Indeed, the widespread industrial use of carcinogenic arylamines has made it apparent that the epidemiology of occupational bladder cancer is for all practical purposes synonymous with the epidemiology of arylamine-induced cancers (*6*).

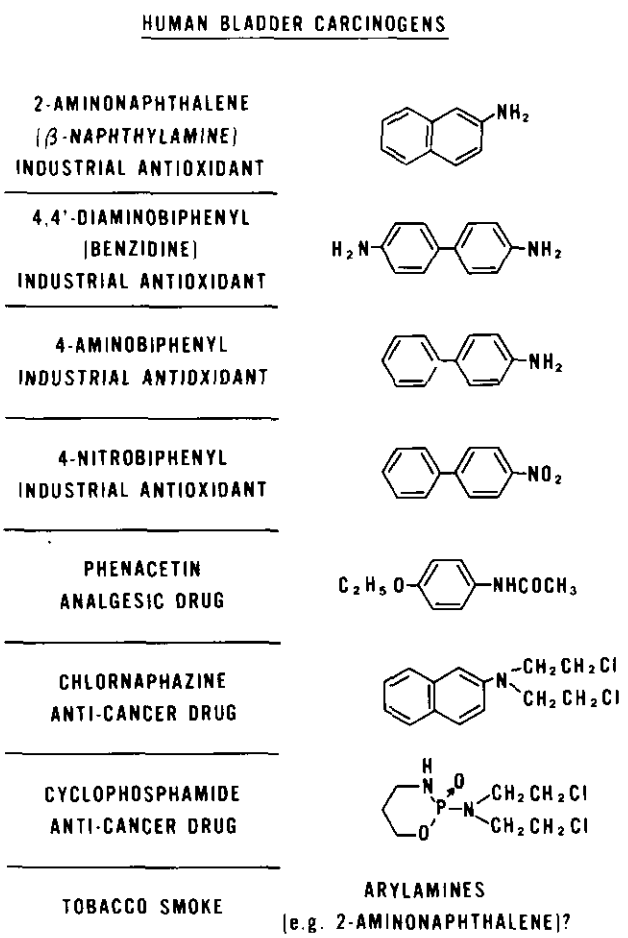

FIGURE 1. Urinary bladder carcinogens shown to represent human hazard.

Estimations of the magnitude of occupational involvement in bladder cancer incidence have ranged from 10 to 50% (*8*), although these values are dynamic and dependent on local environments. For example, epidemiologic case control investigations conducted in the metropolitan Boston area indicate that 15–20% of bladder cancers can be attributed to occupational exposures (*9*), whereas in the metropolitan area of Leeds, England, occupational involvement may be as high as 30% (*10*, *11*), with both areas displaying noteable increases in mortality rates over the past 30 years. Accordingly, a number of high risk occupations have been identified, including chemical, dye, textile, and rubber workers, painters, and hairdressers (*4*, *9*).

Similarly, case control investigations indicate that a variable portion of human bladder cancers can be attributed to exposures to cigarette smoke, the magnitude of involvement reaching 35–40% in metropolitan Boston, and perhaps even higher in metropolitan areas of Canada (*12*, *13*). Common ground is provided here by virtue of the presence in tobacco smoke condensate of various arylamines including the established human bladder carcinogen, 2-aminonaphthalene (*14*), metabolites of which have reportedly been identified in the urine of heavy smokers (*15*). In view of these considerations, it seems reasonable to suggest that in the metropolitan Boston area, for example, perhaps 50–60% of human bladder cancers can be attributed to occupational and cultural situations associated with exposures to various arylamines, many of which represent established human carcinogens.

It is generally accepted that the carcinogenic arylamines represent latent biological arylating agents which require metabolic transformation to chemically reactive metabolites (electrophiles) by enzyme systems of the susceptible host, and that the substrate specificity and functional capacity of enzyme systems involved in activation and detoxication often play major roles as determinants of species and tissue susceptibility (*16–18*). In the dog, for example, susceptibility to bladder carcinogenesis can be correlated with the functional capacity of endoplasmic reticulum-associated mixed function oxygenase enzyme systems requiring NADPH and molecular oxygen and involved in *N*-hydroxylation (activation) to proximate *N*-

hydroxyarylamines. Thus, arylamines which are readily *N*-hydroxylated (e.g., 2-aminonaphthalene) display potent carcinogenic activity in the dog, while closely related arylamines which are not readily *N*-hydroxylated (e.g., 1-aminonaphthalene) are essentially devoid of carcinogenic activity in this species (*19, 20*). Similarly, species such as the guinea pig, which are deficient in the capacity to *N*-hydroxylate aryl nitrogen compounds, are refractory to such insult (*21*).

Such clean simple relationships are, however, often overridden and obscured from view by competing metabolic factors. With the arylamines, this results from the interrelationships of metabolic pathways involved in arylamine activation and detoxication, and the conversion of arylamines to differing ultimate electrophiles with differing tissue specificities and toxicities. Thus, while the proximate carcinogenic metabolites involved in urinary bladder carcinogenesis are evidenced to be nonacetylated *N*-hydroxyarylamines (arylhydroxylamines), the proximate carcinogenic metabolites involved in hepatocarcinogenesis are evidenced to be acetylated *N*-hydroxylarylacetamides (arylhydroxamic acids), with both of these proximate carcinogenic metabolites being derived from arylamines and arylacetamides by virtue of parallel divergent metabolic pathways (Fig. 2) (*22, 23*).

For example, administration of a variety of carcinogenic arylamines to dogs has resulted in the formation of only urinary bladder tumors, the liver apparently being refractory in keeping with an apparent total deficiency in the capacity to *N*-acetylate arylamines, observations providing an indication that *N*-acetylation is not required for bladder carcinogenesis (*23*). In contrast, administration of structurally analogous carcinogenic arylacetamides to dogs has resulted in the unequivocal formation of both urinary bladder tumors and hepatomas, with susceptibility to bladder carcinogenesis being correlated with the substrate specificity of arylacetamide deacetylase enzyme systems, observations providing an indication that removal of the acetyl group is required for bladder carcinogenesis (*24*).

In other words, *N*-hydroxylating enzyme systems can be viewed as components of activation pathways with respect to both arylamine bladder carcinogenesis and arylacetamide hepatocarcinogenesis, while *N*-acetyltransferase enzyme systems can be viewed as components of activation pathways with respect to arylamine hepatocarcinogenesis and as components of detoxication pathways with respect to arylamine bladder carcinogenesis. Similarly, arylacetamide deacetylase enzyme systems can be viewed as components of

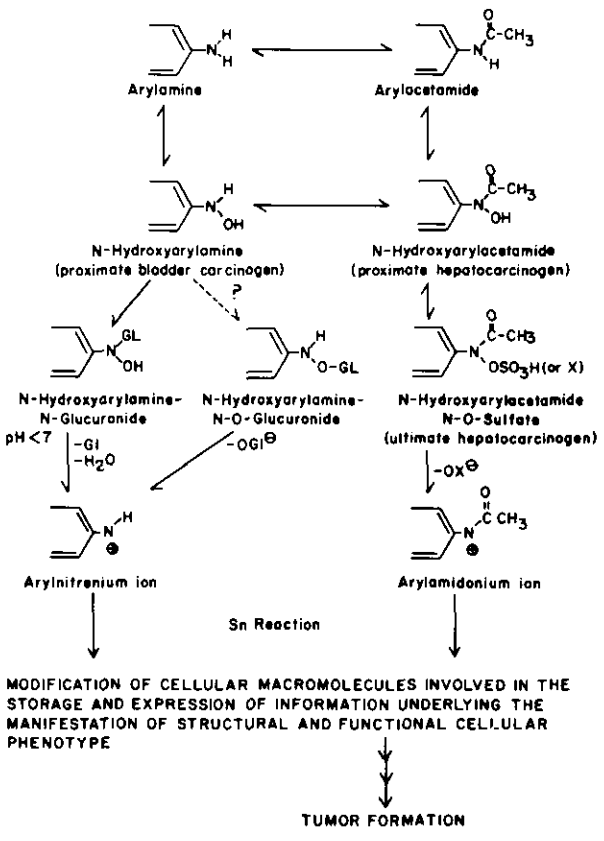

FIGURE 2. Proposed parallel pathways for the metabolic activation of arylamines and arylacetamides to electrophilic reactants involved in urinary bladder and hepatocarcinogenesis.

detoxication pathways with respect to arylacetamide hepatocarcinogenesis and as components of activation pathways with respect to arylacetamide bladder carcinogenesis. These concepts appear to be generally applicable to mammalian systems with the potentially rate-limiting role of a given enzyme system being a relative one, dependent on species-specific metabolic capabilities (*24*). Thus, it is probable that activating and detoxifying enzyme systems might also act as partial determinants of human susceptibility to bladder carcinogenesis by arylamines, and it becomes important to clarify the role of these metabolic factors from a human frame of reference.

For example, in the human, hepatic *N*-acetyltransferase enzyme systems are subject to Mendelian genetic regulation as an autosomal recessive trait, resulting in an approximate 50:50 polymorphic distribution in North American white populations, with individuals displaying either the "slow acetylator" phenotype or the "rapid acetylator" phenotype (*25, 26*). At present, the literature contains a number of entries demonstrating

the role of *N*-acetyltransferase phenotype as a partial determinant of susceptibility to the dose-related toxicities of various acetylable nitrogen compounds (*26–37*) (Table 1). In the case of peripheral neuropathies associated with isoniazid (*27, 28*), phenelzine (*29*), hydralazine (*30*), and salicylazosulfapyridine (*31*) exposures, and as would be expected for bladder cancer associated with arylamine exposures, it is the genetically distinct slow acetylator phenotype demonstrating enhanced susceptibility to toxicity, due in part to the decreased ability of these individuals to detoxify these chemicals by *N*-acetylation. As might be anticipated, the converse is also true. In the case of hepatotoxicity which appears to be elicited by acetylated metabolites (*32*) associated with isoniazid exposure (*33*), it is the genetically distinct rapid acetylator phenotype demonstrating enhanced susceptibility (*34*).

Investigation of the *in vitro* acetylation of arylamines by human liver cytosol has indicated that the carcinogenic arylamines such as 2-aminonaphthalene and 4-aminobiphenyl display a strong affinity for this polymorphically distributed enzyme system, and, moreover, liver cytosol from rapid acetylator phenotypes effected an 8 to 12 times greater acetylation rate *in vitro* than that observed with liver cytosol from slow acetylator phenotypes (*38*). These observations again suggest that rapid and slow acetylator phenotypes might show differential susceptibility to arylamine carcinogenesis in parallel with their differential ability to detoxify arylamines by *N*-acetylation.

Insofar as individuals of the slow acetylator phenotype would be expected to be at relatively greater risk to arylamine carcinogenesis, one would expect a population of bladder cancer patients (provided some portion of patients have arylamine-induced disease) to display a greater percentage of slow acetylator phenotypes than would a control cancer-free population. The results of preliminary efforts to examine this hypothesis in populations of Danish and Swedish urinary bladder cancer patients comprise the subject of this report.

Methods

Patient and Control Populations

Patient populations consisted of individuals with histologically-confirmed papillomas and invasive carcinomas of the urinary bladder. The Swedish population was derived from the rural area surrounding Lund, Sweden and was represented by 115 patients admitted to the Department of Urology at University Hospital. Age-adjusted incidence rates for the counties in this area ranged from 15 to 20 cases/100,000 for 1971 (*39*) and are similar to the incidence rate of 21.2 per 100,000 observed in rural Denmark for the years 1968–72 (*40*).

In contrast, the Danish population was derived from the urban center of Copenhagen, Denmark and was represented by 71 patients admitted to the Department of Urology at Hvidovre University Hospital. The age-adjusted incidence rate for this urban area was estimated at 43.8 per 100,000 for the years 1968–72 and has shown a 100% increase since 1948 (*40*).

Control populations were derived from healthy hospital personnel and hospital patients admitted for non-malignant disease and were represented by 118 individuals from University Hospital in Lund and 74 individuals from Hvidovre University Hospital in Copenhagen. Separate nationality-matched control groups were required in each geographic area due to variability in the percentage of slow acetylator phenotypes with genetic background, although, as an autosomal recessive trait, *N*-acetyltransferase phenotype is not linked to sex and development age (*25*).

Table 1. *N*-Acetyltransferase phenotype as a determinant of human susceptibility to the dose-related toxicities of polymorphically-acetylated nitrogen compounds.

Polymorphically acetylated nitrogen compounds	Dose-related toxicities (susceptible phenotype)	References
Isoniazid	Peripheral neuropathies (S)	Hughes (*27*)
Isoniazid	Peripheral neuropathies (S)	Devadatta (*28*)
Phenelzine	Drowsiness, dizziness, nausea (S)	Evans (*29*)
Hydralazine	Peripheral neuropathies (S) (Lupus erythromatosus-like syndrome)	Perry (*30*)
Salicylazosulfapyridine (Sulfapyridine)	Cyanosis, hemolysis, and reticulocytosis (S)	Das (*31*)
Isoniazid	Hepatitis (R)	Mitchell (*34*)
Procainamide	Systemic lupus erythromatosus (S)	Woosley (*35*)
?	"Spontaneous" systemic Lupus Erythromatosus (S)	Reidenberg (*36*)
?	Diabetic neuropathy (S)	McLaren (*37*)

Clinical Methods

The method employed for determination of human *N*-acetyltransferase phenotype was essentially that of Weber and Brenner (*41*). Patients were instructed not to eat after midnight or to drink fluids after 7 AM of the day of the test. At 9 AM, the subjects were administered 10 mg/kg commercially available sulfamethazine (free acid) orally as a slurry in 2–3 oz of water. After 11 AM, patients were permitted to eat and drink as usual. The bladder was voided of urine at noon and urine was then retained until collection of blood and urine specimens. At 1:30 PM (4.5 hr after sulfamethazine ingestion), samples of blood from a venipuncture and urine were collected and refrigerated, following removal of 0.1 ml aliquots of each which were placed upon filter paper discs (Whatman No. 3) and air dried for analysis.

Free (unacetylated) and total (unacetylated and acetylated) sulfamethazine on filter paper discs were quantitated by a micromodification of the Bratton-Marshall procedure as described by Weber and Brenner (*41*) and phenotypic categorization was accomplished by plotting percent acetylated sulfamethazine in blood versus urine, a manipulation yielding two distinct nonoverlapping populations (Fig. 3).

Statistical p values were determined by the exact method for 2×2 contingency tables and relative risk was calculated as described by Armitage (*42*).

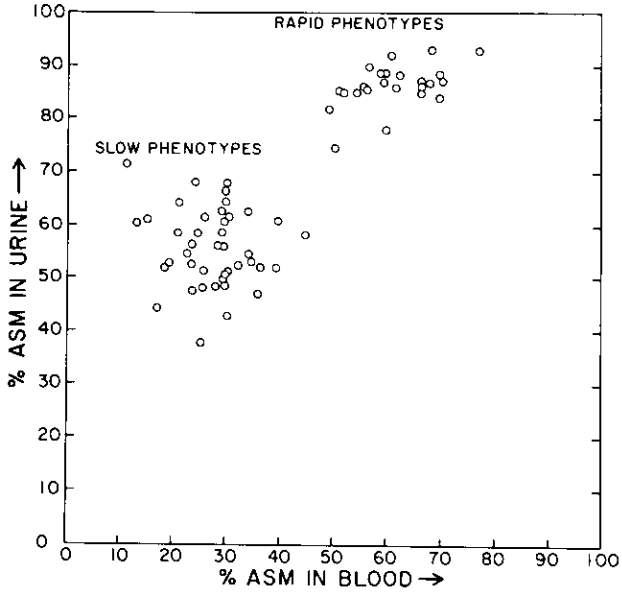

FIGURE 3. Distribution of *N*-acetyltransferase phenotype in a urinary bladder cancer population from Copenhagen, Denmark.

Results

The results of efforts to determine the *N*-acetyltransferase phenotype distribution in bladder cancer and control populations from Denmark and Sweden are presented in Table 2. Individuals in these studies could be readily catagorized as slow and rapid acetylators with slow acetylators demonstrating an average acetylsulfamethazine content of 22.5% in blood and 59.6% in urine and with rapid acetylators demonstrating an average acetylsulfamethazine content of 57.5% in blood and 85.9% in urine.

The slow acetylator phenotype in an urban control population from Copenhagen, Denmark was found to comprise 51.4% (38/74) while in an urban bladder cancer population from the same area, the slow acetylator phenotype was found to comprise 64.8% (46/71) (Fig. 3). With a one-sided test, the excess of 13.4% slow acetylators in the bladder cancer population is characterized by an exact p-value of 0.065, and based upon these data the calculated relative risk for slow acetylator phenotypes is 1.74. Within the bladder cancer population, 95.7% (22/23) of rapid acetylators and 95.5% (42/44) of slow acetylators reported histories of cigarette smoking. In this population, only three patients were nonsmokers, and no information was available on four patients. Similarly, the percent heavy smokers (>1 pack/day, 20%) and light smokers (<1 pack/day, 76%) was similar for both rapid and slow acetylators, and it was not possible to separate out smoking factors.

In contrast, the slow acetylator phenotypes in a rural control population from Lund, Sweden was found to comprise 66.9% (79/118), and in a rural bladder cancer population from the same area, the slow acetylator phenotypes comprised 69.6% (80/115) (Table 2). This differential of 2.7% is not statistically significant, nor do the values of both control and bladder cancer populations differ from control values of 67% previously observed for U. S. Scandinavians (*43*).

Discussion

The finding of an excess of individuals of the slow acetylator phenotype within an urban bladder cancer population from Denmark suggests that arylamines may play a role in disease etiology in this locale and that slow acetylator individuals may be at higher risk to arylamine-induced bladder cancer. Estimations of the percent of urinary bladder cancer in Copenhagen attributable to occupational and smoking factors are presently unavailable, although the high and increasing incidence

Table 2. *N*-Acetyltransferase phenotype distribution in urinary bladder cancer and control populations in Denmark and Sweden.

	Population	Number	ASM in blood, % (mean ± SD)[a]	ASM in urine, % (mean ± SD)[a]	Slow acetylator phenotypes, %	*p* value (relative risk)
Denmark (urban)	Control Slow phenotypes	38	22.8 ± 8.4	62.7 ± 7.2	51.4	0.065 (1.74)
	Control Rapid phenotypes	36	61.1 ± 10.9	86.9 ± 5.5		
	Bladder cancer Slow phenotypes	46	27.1 ± 7.8	54.9 ± 10.8	64.8	
	Bladder cancer Rapid phenotypes	25	60.8 ± 7.2	86.5 ± 4.0		
Sweden (rural)	Control Slow phenotypes	79	20.6 ± 8.9	58.0 ± 8.4	66.9	N.S.
	Control Rapid phenotypes	39	56.6 ± 13.3	84.8 ± 5.5		
	Bladder cancer Slow phenotypes	80	19.6 ± 5.6	62.9 ± 7.3	69.6	
	Bladder cancer Rapid phenotypes	35	51.4 ± 13.2	85.5 ± 5.2		

[a] ASM = acetylsulfamethazine.

rate in this area (*40*) and the known involvement of occupational and smoking factors in urban areas of the U. S. (*44*) indicate that arylamines are likely to play some finite role.

In contrast, the similar distribution of individuals of the slow acetylator phenotype in control and urinary bladder cancer populations from rural Sweden may be interpreted as negative data or as a relative lack of involvement of arylamines in the etiology of rural bladder cancer. Indeed, multifactorial chemical hypotheses, not involving occupational arylamines, have been presented specifically to approach the etiology of those urinary bladder cancers presently referred to as "spontaneous" and in those relatively rural areas providing a more generalized "background" mortality rate (*45*).

The high percentage of slow acetylator phenotypes in control Swedish populations points out a limitation of this approach, insofar as high control values would make more difficult the detection of a significantly higher value in urinary bladder cancer populations. Similarly, these observations point out the variability of *N*-acetyltransferase phenotype distribution as a function of genetic background (*43*). Thus, for example, the percentage of individuals of slow acetylator phenotype ranges from 50% in North American white populations to 70% in Israeli populations, while in oriental populations this recessive trait is much less frequent and appears in only 5–15% of individuals (*43*).

With quantitative involvement of arylamines in disease etiology one might expect about 80% of individuals suffering arylamine toxicities to display the slow acetylator phenotype (*30, 31*), with the percent slow acetylator phenotype in a given bladder cancer population being some function of the percent of patients with arylamine-induced disease. For example, in North American white populations, the slow acetylator phenotype observed in bladder cancer populations might be expected to range between 50 and 80%, depending on whether arylamine involvement ranges from negligible to essentially quantitative. Given this situation, it can be estimated by direct proportion that the detection of a 15% excess of slow acetylator phenotypes in a bladder cancer population would require the involvement of arylamines to the extent of 40% or more. In other words, populations of "spontaneous" bladder cancer patients would be less likely to show such a correlation than would an industrial population with documentable arylamine exposure.

Consequently, confirmation of this hypothesis will require examination of industrial bladder cancer populations in an effort to obtain an empirical estimate of relative risk for slow and rapid acetylator phenotypes. Provided that this arylamine-specific human enzyme system plays a sufficiently rate-limiting role in arylamine carcinogenesis, such investigations ought allow assessment of the feasibility of utilizing this approach in the

determination of high and low risk individuals within high risk environments, and assessment of the relative importance of internal and external factors in the determination of overall relative risk.

In this respect, enzyme systems involved in arylamine *N*-hydroxylation are equally likely to serve as partial determinants of human susceptibility to arylamine-induced urinary bladder cancer. It is important to note here that proximate *N*-hydroxyarylamines involved in bladder carcinogenesis and proximate *N*-hydroxyarylacetamides involved in hepatocarcinogenesis, while more highly carcinogenic than their respective parent arylamines and arylacetamides, are not particularly chemically reactive *per se* and may require further metabolic activation to electrophilic forms. A number of detailed studies now indicate that a second enzyme-mediated process required in the metabolic activation of hepatocarcinogenic arylacetamides involves the esterification of the *N*-hydroxyarylacetamides (*16*, *46*). Furthermore, in the case of sulfate conjugation, there is a close correlation of the activity of soluble sulfotransferase enzyme systems with the susceptibility of experimental animals to hepatocarcinogenesis (*47*). By analogy, one might expect that esterification of *N*-hydroxyarylamines would give rise to electrophilic metabolites. Indeed, the *N*-O-glucuronide conjugate of *N*-hydroxy-2-aminofluorene, when generated *in vitro*, represents an extremely potent electrophile (*48*, *49*), although such compounds have yet to be demonstrated as *in vivo* metabolites. Alternatively, the *N*-glucuronides of *N*-hydroxyarylamines (Fig. 2), which are readily formed by dog and human hepatic microsomal enzyme systems, undergo hydrolysis at the acidity of urine to yield similar electrophilic reactants (*50*).

Thus, while the exact identity of the ultimate carcinogenic metabolite(s) involved in the initiation of bladder carcinogenesis remains uncertain, it seems reasonable to expect that any enzyme system involved in the further metabolic activation of *N*-hydroxyarylamines might serve as a potential determinant of bladder susceptibility. Indeed, full assessment of relative risk to arylamine carcinogenesis might be expected to require assessment of external factors such as age of initial exposure, relative exposure levels, and duration of exposure; and assessment of internal factors such as the relative ratio of the functional capacities of activation pathways to detoxication pathways.

The basis for these approaches to the assessment of the role of internal factors in the determination of relative risk is derived from the examination of chemical carcinogenesis in experimental animal models and rests upon the observation and realization that many chemical carcinogens, including the arylhydrocarbons and arylamines, are subject to host enzyme-mediated activation and detoxication processes, and that the substrate specificity and functional capacity of these enzyme systems often play major roles as determinants of species and tissue susceptibility (*16*, *17*, *47*).

In the examination of causality and relative risk in the human population, this approach is exemplified by recent successful efforts to correlate differential activities of arylhydrocarbon hydroxylase (AHH, an enzyme system involved in arylhydrocarbon activation processes) with differential human susceptibility to cigarette smoke (presumably arylhydrocarbon)-induced lung and laryngeal cancer (*51*, *52*). Such approaches represent efforts to make molecular level observations demonstrating differentials in the specificity and functional capacity of host-mediated activation and detoxication processes that are analogically and spatiotemporally consistent with both cellular and organismal level observations of risk, an approach for which the term "molecular epidemiology" has been coined (*53*). Thus, molecular epidemiology concerns itself with causal disease processes including those processes involved in the emergence of environmental hazard, those processes involved in the environmental dynamics of causal agents, and those processes involved in causal agent-host interactions which underly the initiation of effectual disease processes (*45*).

With respect to urinary bladder cancer causality, these approaches have included: (1) the identification of causal agents in high risk environments as exemplified by the identification of carcinogenic arylamines in situations involving occupational exposures (*3*, *4*) and the identification of 2-aminonaphthalene in cigarette smoke (*14*); (2) the identification of causal agents in biologic fluids derived from the potential host as exemplified by the identification of diazotizable arylamines in the urine of exposed workmen (*4*) and the identification of 2-nitrosonaphthalene in the urine of heavy smokers (*15*); and (3) the identification of host metabolic factors involved in the attenuation of causal agent-host interactions and the correlation of differentials in rates of activation and detoxication with relative risk as exemplified by the present report and reports of an excess activity of arylhydrocarbon hydroxylase among individuals with previous smoking histories and lung cancer (*51*, *52*).

Thus, these approaches in molecular epidemiology represent efforts to make empirical observations of differential molecular-level attenuations of causal agent-host interactions that are analogically consistent with epidemiologic observations of risk

at the organismal level, and represent efforts to determine the true propositions of one level of organization and observation (organismal) by the making of analogical deductions based upon observations derived from underlying levels of organization (molecular) (*45*, *54*). Insofar as the majority of human epithelial cancers bear evidence of chemical etiology (*45*, *53*), it is reasonable to extend these modes of inquiry to the human situation.

The authors would like to acknowledge the technical assistance of Ms. Ruth Lowengart. Recognition is also due to Ms. Mary Post and Ms. Jean Ann Boldon for assistance in the preparation of the manuscript.

This research was supported by U. S. Public Health Service Grant CA-14524 from the National Bladder Cancer Project, National Cancer Institute.

REFERENCES

1. Rehn, L. Blasengeschwültse bei fuchsin-arbeitern. Arch. Klin. Chir. 50: 588 (1895).
2. Hueper, W. C., Wiley, F. H., Wolfe, H. D., et al. Experimental production of bladder tumors in dogs by administration of beta-naphthylane. J. Ind. Hyg. Toxicol. 20: 46 (1938).
3. Case, R. A. M., and Hosker, M. E. Tumors of the urinary bladder in workmen engaged in the manufacture and use of certain dyestuff intermediates in the British chemical industry. Part I. The role of aniline, benzidine, alpha-naphthylamine, and beta-nephthylamine. Brit. J. Ind. Med. 11: 75 (1954); Brit. J. Prev., Soc. Med. 8: 39 (1954).
4. Hueper, W. C. Occupational and Environmental Cancer of the Urinary System. Yale University Press, New Haven, 1969, p. 465.
5. Cole, P. Lower urinary tract. In: Cancer Epidemiology and Prevention: Current Concepts. D. Schottenfield, Ed., Charles C Thomas, Springfield, Illinois, 1974, pp. 233-262.
6. Parkes, H. G. The epidemiology of the aromatic amine cancers. In: Chemical Carcinogens (Am. Chem. Soc. Monograph 173), C. E. Searle, Ed., American Chemical Society, Washington, D. C., 1976, p. 462.
7. Stula, E. F., Barnes, J. R., Sherman, H., Reinhardt, C. F., and Zapp, J. Urinary bladder tumors in dogs from 4,4'-methylene-bis(2-chloroaniline) (MOCA). J. Environ. Pathol. Toxicol. 1: 1 (1977).
8. Higginson, J. Chronic toxicology—an epidemiologist's approach to the problem of carcinogenesis. Essays. Toxicol. 77: 29 (1976).
9. Cole, P., Hoover, R., and Friedell, G. H. Occupation and cancer of the lower urinary tract. Cancer 29: 1250 (1972).
10. Anthony, H. M., and Thomas, G. M. Tumors of the urinary bladder: An analysis of the occupations of 1,030 patients in Leeds, England. J. Natl. Cancer Inst. 45: 879 (1970).
11. Cole, P., and Hoover, R. Comments on: tumors of the urinary bladder: An analysis of the occupations of 1,030 patients in Leeds, England, Anthony, H. and Thomas, G. J. Natl. Cancer Inst. 46: 1111 (1971).
12. Cole, P., Monson, R. R., Haning, H., and Friedell, G. H. Smoking and cancer of the lower urinary tract. New Engl. J. Med. 284: 129 (1971).
13. Miller, A. B. The etiology of bladder cancer from the epidemiological viewpoint. Cancer Res. 37: 2939 (1977).
14. Hoffman, D., Masuda, Y., and Wynder, E. L. α-Naphthylamine and β-naphthylamine in cigarette smoke. Nature 221: 254 (1969).
15. Radomski, J. Environmental bladder carcinogens. National Bladder Cancer Project, Investigators' Workshop. January 25-27, 1976, p. 20.
16. Miller, J. A., and Miller, E. C. The metabolic activation of carcinogenic aromatic amines and amides. Progr. Exptl. Tumor Res. 11: 273 301 (1969).
17. Weisburger, J. H., and Weisburger, E. K. Biochemical formation and pharmacological, toxicological, and pathological properties of hydroxylamines and hydroxamic acids. Pharmacol. Rev. 25: 1 (1973).
18. Clayson, D. B., and Garner, R. C. Carcinogenic aromatic amines and related compounds. In: Chemical Carcinogens (Am. Chem. Soc. Monograph 173), C. E. Searle, Ed., American Chemical Society, Washington, D. C., 1976, p. 366.
19. Radomski, J. L., and Brill, E. Bladder cancer induction by aromatic amines: Role of *N*-hydroxy metabolites. Science 167: 992 (1970).
20. Radomski, J. L., Brill, E., Deichmann, W. B., and Glass, E. M. Carcinogenicity testing of *N*-hydroxy and other oxidation and decomposition products of 1- and 2-naphthylamine. Cancer Res. 31: 1461 (1971).
21. Miller, E. C., Miller, J. A., and Enomoto, M. The comparative carcinogenicities of 2-acetylaminofluorene and its *N*-hydroxy metabolite in mice, hamsters, and guinea pigs. Cancer Res. 23: 2018 (1964).
22. Poirier, L. A., Miller, J. A., and Miller, E. C. The *N*- and ring-hydroxylation of 2-acetylaminofluorene and the failure to detect *N*-acetylation of 2-aminofluorene in the dog. Cancer Res. 23: 790 (1963).
23. Lower, G. M., Jr., and Bryan, G. T. Enzymatic *N*-acetylation of carcinogenic aromatic amines by liver cytosol of species displaying different organ susceptibilities. Biochem. Pharmacol. 22: 1581 (1973).
24. Lower, G. M., Jr., and Bryan, G. T. Enzymic deacetylation of carcinogenic arylacetamides by tissue microsomes of the dog and other species. J. Toxicol. Environ. Health 1: 421 (1976).
25. Evans, D. A. P., and White, T. A. Human acetylation polymorphism. J. Lab. Clin. Med. 63: 394 (1964).
26. Drayer, D. E., and Reidenberg, M. M. Clinical consequences of polymorphic acetylation of basic drugs. Clin. Pharm. Therap. 22: 251 (1977).
27. Hughes, H. B., Biehl, J. P., Jones, A. P., and Schmidt, L. H. Metabolism of isoniazid in man as related to the occurrence of peripheral neuritis. Am. Rev. Tuberculosis 70: 266 (1954).
28. Devadatta, S., Gangadharam, P. R. J., Andrews, R. H., Fox, W., Ramakrishnan, C. V., Selkon, J. B., and Velu, S. Peripheral neuritis due to isoniazid. Bull. WHO 23: 587 (1960).
29. Evans, D. A. P., Davidson, K., and Pratt, R. T. C. The influence of acetylator phenotype on the effects of treating depression with phenelzine. Clin. Pharmacol. Therap. 6: 430 (1965).
30. Perry, H. M., Jr., Sakamoto, A., and Tan, E. M. Relationship of acetylating enzyme to hydralazine toxicity. J. Lab. Clin. Med. 70: 1020 (1967).
31. Das, K. M., Eastwood, N. A., McManus, J. P. A., and Sircus, W. Adverse reactions during salicylazosulfapyridine therapy and the relation with drug metabolism and acetylator phenotype. New Engl. J. Med. 289: 491 (1973).
32. Nelson, S. D., Mitchell, J. R., Timbrell, J. A., Snodgrass, W. R., and Corcoran, G. B. Isoniazid and ipromiazid: Activation of metabolites to toxic intermediates in man and rat. Science 193: 901 (1976).
33. Black, M., Mitchell, J. R., Zimmerman, H. J., Ishak, K. G., and Epler, G. R. Isoniazid-associated hepatitis in 114 patients. Gastroenterol. 69: 289 (1975).

34. Mitchell, J. R., Thorgeirsson, U. P., Black, M., Timbrell, J. A., Snodgrass, W. R., Potter, W. Z., Jollow, D. J., and Keiser, H. R. Increased incidence of isoniazid hepatitis in rapid acetylators: Possible relation to hydrazine metabolites. Clin. Pharmacol. Therap. 18: 70 (1975).
35. Woosley, R. L., Nies, A. S., Drayer, D., Reidenberg, M., and Oates, J. A. Acetylator phenotype as a factor in procainamide-induced lupus erythematosus. Clin. Res. 25: 279 A (1977).
36. Reidenberg, M. M., and Martin, J. H. The acetylator phenotype of patients with systemic lupus erythematosus. Drug. Metab. Disposition 2: 71 (1974).
37. McLaren, E. H., Burden, A. C., and Moorhead, P. J. Acetylator phenotype in diabetic neuropathology. Brit. Med. J. 2: 291 (1977).
38. Glowinski, I., Radtke, H. E., and Weber, W. W. Genetic susceptibility to chemical carcinogenesis from aromatic amines. Pharmacol. 18: 231 (1976).
39. Anonymous. Cancer Incidence in Sweden 1971. National Board of Health and Welfare, the Cancer Registry, S-106 30 Stockholm, Sweden, 1975.
40. Clemmesen, J. Statistical Studies in the Aetiology of Malignant Neoplasms. Vol. V. Trends and Risks, Denmark 1943-1977. Supplement 261, Acta Pathologica et Microbiologica Scandinavica, Munksgaard, Kobenhavn, 1977, pp 286.
41. Weber, W. W., and Brenner, W. A filter paper method for determining isoniazid acetylator phenotype. Am. J. Human Genet. 26: 467 (1974).
42. Armitage, P. Statistical Methods in Medical Research. Wiley, New York, 1973, pp. 427-433.
43. Harris, H. W., Knight, A., and Selin, M. J. Comparison of isoniazid concentrations in the blood of people of Japanese and European descent—therapeutic and genetic implications. Am. Rev. Tuberc. Pulm. Dis. 78: 944 (1958).
44. Hoover, R., Mason, T. J., McKay, F. W., and Fraumeni, J. F., Jr. Cancer by county: New resource for etiologic clues. Science 189: 1005 (1975).
45. Lower, G. M., Jr., and Bryan, G. T. Carcinogenesis and etiology: natural systems approaches to causality and control. In: Management of Urologic Cancers, N. Javadopour, Ed., Williams and Wilkins, Baltimore, 1978, Chap. 2.
46. King, C. M., and Phillips, B. Enzyme-catalyzed reactions of the carcinogen N-hydroxy-2-fluorenylacetamide with nucleic acid. Science 159: 1351 (1968).
47. DeBaun, J. R., Miller, E. C., and Miller, J. A. *N*-Hydroxy-2-acetyl-aminofluorene sulfotransferase: Its probable role in carcinogenesis and in protein-(methion-5-yl) binding in rat liver. Cancer Res. 30: 577 (1970).
48. Irving, C. C., and Russell, L. T. Synthesis of the *o*-glucuronide of *N*-2-fluorenylhydroxylamine. Reaction with nucleic acids and with guanosine 5-monophosphate. Biochemistry 9: 2471 (1970).
49. Cardona, R. A., and King, C. M. Activation of the *o*-glucuronide of the carcinogen *N*-hydroxy-FAA by enzymatic deacetylation *in vitro:* Formation of FA-tRNA adducts. Biochem. Pharmacol. 25: 1051 (1976).
50. Kadlubar, F. F., Miller, J. A., and Miller, E. C. Hepatic microsomal *N*-glucuronidation and nucleic acid binding of *N*-hydroxy arylamines in relation to urinary bladder carcinogenesis. Cancer Res. 37: 805 (1977).
51. Kellermann, G., Shaw, C. R., and Kellermann, M. L. Aryl hydrocarbon hydroxylase inducibility and bronchogenic carcinoma. New Engl. J. Med. 289: 934 (1973).
52. Kellerman, G., Kellerman, M. L., Jett, J. R., Moses, H. L., and Fontana, R. S. Aryl hydrocarbon hydroxylase in man and lung cancer. Human Genetics (Suppl. 1) 161 (1978).
53. Higginson, J. The role of the pathologist in environmental medicine and public health. Am. J. Pathol. 86: 460 (1967).
54. Brody, H. The systems view of man: implications for medicine, science and ethics. Perspect. Biol. Med. 17: 71 (1973).